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SEQUENCE LISTING

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Helliwell, Christopher Andrew  
Waterhouse, Peter Michael

<120> Efficient gene silencing in plants using short dsRNA sequences

<130> 021565-156

<140> US 10/780,638  
<141> 2004-02-19

<150> US 60/447,711  
<151> 2003-02-19

<160> 33

<170> PatentIn version 3.1

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Arabidopsis thaliana var. Landsberg erecta

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<223> PolIII promoter region

<220>  
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tctcttcttc tatcttaaat ctgtgtatac tatttcacta ttgggctttt tattagtcta    180
taatgggact caaaataagg ctttggtcca catcaaaaag ataagtcaca aatcaaaaact    240
aaattcagag tcttttctcc cacatcggtc actgtactca ttttgtgttt gtttatatat    300
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        thaliana var. Landsberg erecta including 86 bases downstream
        of the transcription initiation site.

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<223> XhoI restriction site

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<220>
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<223> poly T stretch

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<220>
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<223> SalI restriction site

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<220>
<221> misc_feature
<222> (7)..(408)
<223> PolIII promoter region.

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<400> 2

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ctcgagatgt tgttggtacc agaaagtaaa taaatgttca atctctgatg ttctcaagta    60
agtgagtttt attgggaata atattaactt atgttcttct tgcatttgat ttctttgccg    120
ctctcttctt ctatcttaaa tctgtgtata ctatttcact attgggcttt ttattagtct    180
ataatgggac tcaaaataag gctttggccc acatcaaaaa gataagtcac aaatcaaaac    240
taaattcaga gtcttttctc ccacatcggt cactgtactc ttttgtgttt gtttatatat    300
tacacgaacc gatctttggt acgtcgagct aagtaacatg agcttgtaac ccatgtgggg    360
acattaagat ggtggaacac tggctcgggt ccacgggccg gttctgttgt cgactttttt    420
tttctcgag                                     429

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 var. Landsberg erecta

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 <223> EcoRI restriction site

<220>  
 <221> misc\_feature  
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 <223> PvuI restriction site

<220>  
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<220>  
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 <222> (329)..(334)  
 <223> EcoRI restriction site

<220>  
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 <223> Pol III promoter region

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agatctgttt tggctatggt ggacgaaaca agtgaacttt taggatcaac ttcagtttat	120
atatggagct tatatcgagc aataagataa gtgggctttt tatgtaattt aatgggctat	180
cgtccataga ttcactaata cccatgccca gtacccatgt atgcgtttca tataagctcc	240
taattttctc cacatcgctc aaatctaaac aaatcttggt gtatatataa cactgagga	300
gcaacattgg tcacgatcgt ttttttttga attc	334

<210> 4  
 <211> 467  
 <212> DNA  
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<220>  
 <223> sequence of the promoter of the U3 snRNA gene of Arabidopsis  
 thaliana var. Landsberg erecta including 136 bases downstream  
 of the transcription initiation site.

<220>  
 <221> misc\_feature  
 <222> (1)..(6)  
 <223> EcoRI restriction site

<220>  
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 <222> (7)..(446)  
 <223> Pol III promoter region

<220>

<221> misc\_feature  
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 <223> XhoI restriction site

<220>  
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 <222> (453)..(461)  
 <223> poly T stretch

<220>  
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 <222> (462)..(467)  
 <223> EcoRI restriction site

<400> 4

gaattcttat	gcagcctgtg	atggataact	gaatcaaaca	aatggcgtct	gggtttaaga	60
agatctgttt	tggctatgtt	ggacgaaaca	agtgaacttt	taggatcaac	ttcagtttat	120
atatggagct	tatatcgagc	aataagataa	gtgggctttt	tatgtaattt	aatgggctat	180
cgtccataga	ttcactaata	cccatgcccc	gtacccatgt	atgcgtttca	tataagctcc	240
taattttctc	cacatcgctc	aaatctaaac	aaatcttggt	gtatatataa	cactgagggg	300
gcaacattgg	tcacgacctt	acttgaacag	gatctgttct	ataggctcgt	acctctgttt	360
ccttgatttc	tcaagagaca	ggcccttaac	cctggttgat	gaaccatgac	cgtgaggcta	420
gagcgtgatt	gacggctacg	atcgctctcg	agtttttttt	tgaattc		467

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<220>  
 <223> sequence of the promoter of the U6 snRNA gene of Arabidopsis thaliana var. Landsberg erecta including 3 bases downstream of the transcription initiation site

<220>  
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 <222> (1)..(6)  
 <223> XhoI restriction site

<220>  
 <221> misc\_feature  
 <222> (7)..(436)  
 <223> Pol III promoter region

<220>  
 <221> misc\_feature  
 <222> (437)..(442)  
 <223> SalI restriction site

<220>  
 <221> misc\_feature  
 <222> (443)..(450)  
 <223> poly T stretch

<220>  
 <221> misc\_feature  
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 <223> Sac I restriction site

&lt;400&gt; 5

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ttagcttttt ttcttcttct tcgttcatac agtttttttt tgtttatcag cttacatttt      120
cttgaaccgt agctttcgtt ttcttctttt taactttcca ttcggagttt ttgtatcttg      180
tttcatagtt tgtcccagga ttagaatgat taggcacga accttcaaga atttgattga      240
ataaaacatc ttcatcttta agatatgaag ataactttca aaaggcccct gggaatctga      300
aagaagagaa gcaggcccat ttatatggga aagaacaata gtatttctta tataggccca      360
tttaagttga aaacaatctt caaaagtccc acatcgctta gataagaaaa cgaagctgag      420
tttatataca gctagagtcg actttttttt gagctc                                456

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&lt;210&gt; 6

&lt;211&gt; 488

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the promoter of the U3 snRNA gene of Arabidopsis thaliana var. Landsberg erecta including 20 bases downstream of the transcription initiation site

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (1)..(6)

&lt;223&gt; XhoI restriction site

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (7)..(468)

&lt;223&gt; Pol III promoter region

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (469)..(474)

&lt;223&gt; PvuI restriction site

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (475)..(482)

&lt;223&gt; Poly T stretch

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (483)..(488)

&lt;223&gt; XhoI restriction site

&lt;400&gt; 6

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ctcgagcttc gttgaacaac ggaaactcga cttgccttcc gcacaatata tcatttcttc      60
ttagcttttt ttcttcttct tcgttcatac agtttttttt tgtttatcag cttacatttt      120
cttgaaccgt agctttcgtt ttcttctttt taactttcca ttcggagttt ttgtatcttg      180
tttcatagtt tgtcccagga ttagaatgat taggcacga accttcaaga atttgattga      240
ataaaacatc ttcatcttta agatatgaag ataactttca aaaggcccct gggaatctga      300
aagaagagaa gcaggcccat ttatatggga aagaacaata gtatttctta tataggccca      360
tttaagttga aaacaatctt caaaagtccc acatcgctta gataagaaaa cgaagctgag      420
tttatataca gctagagtcg aagtagtgat tgtcccttcg gggacatccg atcggttttt      480
ttctcgag                                488

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<210> 7  
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 <213> Artificial Sequence

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 <222> (1)..(6)  
 <223> EcoRI restriction site

<220>  
 <221> misc\_feature  
 <222> (7)..(384)  
 <223> Pol III promoter region

<220>  
 <221> misc\_feature  
 <222> (385)..(390)  
 <223> PvuI restriction site

<220>  
 <221> misc\_feature  
 <222> (391)..(399)  
 <223> poly T stretch

<220>  
 <221> misc\_feature  
 <222> (400)..(405)  
 <223> EcoRI restriction site

<400> 7

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aagttatcag gcatgcatgg atcttggagg aatcagatgt gcagtcaggg accatagcac	120
aggacaggcg tcttctactg gtgctaccag caaatgctgg aagccgggaa cactgggtac	180
gttggaacc acgtgatgtg gagtaagata aactgtagga gaaaagcatt tcgtagtggg	240
ccatgaagcc tttcaggaca tgtattgcag tatgggccgg cccattacgc aattggacga	300
caacaaagac tagtattagt accacctcgg ctatccacat agatcaaagc tggtttaaaa	360
gagttgtgca gatgatccgt ggcacgatcg ttttttttgg aattc	405

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 <212> DNA  
 <213> Artificial Sequence

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 <223> sequence of the promoter of the U3 snRNA of tomato (a garden variety with small gourd-shaped yellow fruit)

<220>  
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 <222> (1)..(6)  
 <223> EcoRI restriction site

<220>  
 <221> misc\_feature  
 <222> (7)..(421)  
 <223> Pol III promoter region

<220>  
 <221> misc\_feature  
 <222> (422)..(427)  
 <223> PvuI restriction site

<220>  
 <221> misc\_feature  
 <222> (428)..(436)  
 <223> Poly T stretch

<220>  
 <221> misc\_feature  
 <222> (437)..(442)  
 <223> EcoRI restriction site

<400> 8

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cattattttc agactctact gaagattgaa ttgaatgaga aactatgaaa ctttacaagt	120
gaattattat ggagttcatg gcaactgcta tggagttttt cctactggga attggaacgg	180
tttctacgaa attaactgtc cacacgttaa aaatataaat taatgcgtaa ttgttatttt	240
ttctataaca aataaaaaaac tgaaatacga cataaatttt attactttta ttgcacttta	300
gccttagaga tattgcgttg tagtcggcgt aggtgtgtca ggggccata tattgttccc	360
acatcggcag tgcagcacat aaactctagc gttataagaa tctatccact atcaacggtc	420
acgatcgttt ttttttgaat tc	442

<210> 9  
 <211> 295  
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<220>  
 <223> sequence of the dsRNA encoding region of 94bp for silencing expression of the GUS gene (GUShp94)

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 <223> SalI restriction site

<220>  
 <221> misc\_feature  
 <222> (6)..(11)  
 <223> PvuI restriction site

<220>  
 <221> misc\_feature  
 <222> (12)..(100)  
 <223> GUS sequence (sense)

<220>  
 <221> misc\_feature  
 <222> (101)..(195)

<223> spacer sequence

<220>

<221> misc\_feature

<222> (190)..(195)

<223> BamHI restriction site

<220>

<221> misc\_feature

<222> (196)..(284)

<223> GUS sequence (antisense)

<220>

<221> misc\_feature

<222> (285)..(290)

<223> PvuI restriction site

<220>

<221> misc\_feature

<222> (290)..(295)

<223> SalI restriction site

<400> 9

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gtggtgacgc	atgtcgcgca	agactgtaac	cacgcgtctg	ttgactggca	ggtggtggcc	120
aatggtgatg	tcagcgttga	actgcgtgat	gcggatcaac	aggtggttgc	aactggacaa	180
ggcactagcg	ggatccagac	gcgtgggttac	agtcttgccg	gacatgcgtc	accacggtga	240
tatcgtccac	ccaggtgttc	ggcgtgggtgt	agagcattac	gctgcgatcg	tcgac	295

<210> 10

<211> 93

<212> DNA

<213> Artificial Sequence

<220>

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<220>

<221> misc\_feature

<222> (1)..(6)

<223> SalI restriction site

<220>

<221> misc\_feature

<222> (7)..(42)

<223> GUS sequence (sense)

<220>

<221> misc\_feature

<222> (43)..(51)

<223> spacer sequence

<220>

<221> misc\_feature

<222> (52)..(87)

<223> GUS sequence (antisense)



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<222> (88)..(93)
<223> Sal I restriction site

<400> 10

gtcgactggg cagatgaaca tggcatcgtg gtgattgatg aatgcgagaa cttcatcaat 60
caccacgatg ccatgttcat ctgcccagtc gac 93

<210> 11
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the dsRNA encoding region of 21 bp for silencing
expression of the GUS gene (GUShp21)

<220>
<221> misc_feature
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<223> SalI restriction site

<220>
<221> misc_feature
<222> (7)..(22)
<223> GUS sequence (sense)

<220>
<221> misc_feature
<222> (23)..(28)
<223> spacer sequence

<220>
<221> misc_feature
<222> (29)..(44)
<223> GUS sequence (antisense)

<220>
<221> misc_feature
<222> (45)..(50)
<223> Sal I restriction site

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gtcgactggg cagatgaaca tgtacgatca tggtcatctg cccagtcgac 50

<210> 12
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> sequence of the dsRNA encoding region of 42 bp for silencing
expression of the PHYB gene, derived from the 5' end of PHYB
(PHYB5hp 42)-upper strand

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&lt;400&gt; 12

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tcgacggagt cgggggtagt ggcgggtggcc gtggcggtgg ccgtggagga ggccacggcc 60
accgccacgg ccaccgccac taccgccgac tccg 94
```

&lt;210&gt; 13

&lt;211&gt; 94

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp 42)-lower strand

&lt;400&gt; 13

```
tcgacggagt cgggggtagt ggcgggtggcc gtggcggtgg ccgtggcctc ctccacggcc 60
accgccacgg ccaccgccac taccgccgac tccg 94
```

&lt;210&gt; 14

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp 21)-upper strand

&lt;400&gt; 14

```
tcgacggagt cgggggtagt ggcgggaggag gccgccacta cccccgactc cg 52
```

&lt;210&gt; 15

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the 5' end of PHYB (PHYB5hp 21)-lower strand

&lt;400&gt; 15

```
tcgacggagt cgggggtagt ggcgggcctcc tccgccacta cccccgactc cg 52
```

&lt;210&gt; 16

&lt;211&gt; 94

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 42)-upper strand

&lt;400&gt; 16

```
tcgatggatg gtgtgggttca gccatgtagg gatatggcgg gggaacagga gggttccccc 60
gccatatccc tacatggctg aaccacacca tcca 94
```

&lt;210&gt; 17

&lt;211&gt; 94

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 42 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 42)-lower strand

&lt;400&gt; 17

```
tcgatggatg gtgtgggttca gccatgtagg gatatggcgg gggaaccctc ctgttccccc 60
gccatatccc tacatggctg aaccacacca tcca 94
```

&lt;210&gt; 18

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 21)-upper strand

&lt;400&gt; 18

```
tcgatggatg gtgtgggttca gccataggag gatggctgaa ccacacctcc aa 52
```

&lt;210&gt; 19

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 21 bp for silencing expression of the PHYB gene, derived from the center of PHYB (PHYBChp 21)-lower strand

&lt;400&gt; 19

```
tcgatggatg gtgtgggttca gccatcctcc tatggctgaa ccacaccatc ca 52
```

&lt;210&gt; 20

&lt;211&gt; 94

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> sequence of the dsRNA encoding region of 42 bp for silencing

expression of the PHYB gene, derived from the 3' end of PHYB  
(PHYB3hp 42)-upper strand

<400> 20

tcgacattgt caactgctag tggaagtggg gacatgatgc tgatgaagga ggtcatcagc 60  
atcatgtcac cacttcact agcagttgac aatg 94

<210> 21

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 42 bp for silencing  
expression of the PHYB gene, derived from the 3' end of PHYB  
(PHYB3hp 42)-lower strand

<400> 21

tcgacattgt caactgctag tggaagtggg gacatgatgc tgatgacctc cttcatcagc 60  
atcatgtcac cacttcact agcagttgac aatg 94

<210> 22

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing  
expression of the PHYB gene, derived from the 3' end of PHYB  
(PHYB3hp 21)-upper strand

<400> 22

tcgacattgt caactgctag tggaaggag gttccactag cagttgacaa tg 52

<210> 23

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> sequence of the dsRNA encoding region of 21 bp for silencing  
expression of the PHYB gene, derived from the 3' end of PHYB  
(PHYB3hp 21)-lower strand

<400> 23

tcgacattgt caactgctag tggaacctcc tttccactag cagttgacaa tg 52

<210> 24

<211> 94

<212> DNA

<213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; sequence of the dsRNA encoding region of 42 bp for silencing expression of the PDS gene (PDS42)-upper strand

&lt;400&gt; 24

```
tcgacttaac ttgtaaggaa tattacgata ctaaccgggc aatgctagga ggagcattga    60
ccggtagga tcgtaatat cttacaagt taag                                     94
```

&lt;210&gt; 25

&lt;211&gt; 94

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; sequence of the dsRNA encoding region of 42 bp for silencing expression of the PDS gene (PDS42)-lower strand

&lt;400&gt; 25

```
tcgacttaac ttgtaaggaa tattacgata ctaaccgggc aatgctcctc ctagcattga    60
ccggtagga tcgtaatat cttacaagt taag                                     94
```

&lt;210&gt; 26

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; sequence of the dsRNA encoding region of 21 bp for silencing expression of the PDS gene (PDS21)-upper strand

&lt;400&gt; 26

```
tcgacttaac ttgtaaggaa tattaaggag gtaatatcc ttacaagta ag              52
```

&lt;210&gt; 27

&lt;211&gt; 52

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; sequence of the dsRNA encoding region of 21 bp for silencing expression of the PDS gene (PDS21)-lower strand

&lt;400&gt; 27

```
tcgacttaac ttgtaaggaa tattacctcc ttaatatcc ttacaagta ag              52
```

&lt;210&gt; 28

&lt;211&gt; 115

&lt;212&gt; DNA

&lt;213&gt; Artificial sequence

<220>  
 <223> small hairpin RNA coding region (GUS\_A)

<220>  
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 <222> (1)..(11)  
 <223> SalI/PvuI restriction sites

<220>  
 <221> misc\_feature  
 <222> (12)..(53)  
 <223> sense RNA encoding region

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 <222> (54)..(62)  
 <223> loop structure

<220>  
 <221> misc\_feature  
 <222> (63)..(104)  
 <223> antisense RNA encoding region

<220>  
 <221> misc\_feature  
 <222> (105)..(115)  
 <223> SalI/PvuI restriction sites

<400> 28

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gtcgacgatac gtgcggtcac tcattacggc aaagtgtggg tcaataatca ggagttcctt    60
cttcctgatt attgaccac actttgccgt aatgagtgac cgcagtcgac gatcg      115
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<210> 29  
 <211> 112  
 <212> DNA  
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